Compassionate-use of sorafenib in Flt3-ITD positive acute myeloid leukemia: sustained regression prior and post allogenic stem cell transplantation

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Running title: Sorafenib for relapsed Flt3-ITD+AML prior and post stem cell transplantation
Abstract

AML patients with internal tandem duplication (ITD) mutations in the Fms-like tyrosine-3 (Flt3) gene have a dismal prognosis. Here we report compassionate-use results with the multi-kinase-, and Flt3-ITD-inhibitor sorafenib for the treatment of relapsed or refractory Flt3-ITD-positive AML. Sorafenib induced clinically meaningful and very rapid responses in all six patients treated either prior (n=2), post (n=3), or both, prior and post (n=1) allogenic stem cell transplantation (allo-SCT). Sorafenib-induced remissions facilitated allo-SCT in two of the three refractory patients. Two of the four patients that were treated after allo-SCT survived 216 and 221 days, respectively, while the other two remain in ongoing complete molecular remission. Sorafenib-response was associated with an inhibition of the anti-apoptotic Flt3-ITD target Stat-5 in vivo.

Together, sorafenib-monotherapy prior or post allo-SCT has remarkable clinical activity in poor risk Flt3-ITD-positive AML and deserves further evaluation in prospective clinical trials.
Introduction

Acute myeloid leukemia (AML) is the most frequent acute leukemia of adults and has an unfavorable prognosis 1. In 20-30% of the AML-patients mutations in the Flt3-receptor tyrosine kinase (Flt3-RTK) occur, leading to internal tandem duplications in the juxtamembrane domain of the receptor (Flt3-ITD) 2,3. Flt3-ITD dictates a particularly poor clinical outcome 4-8. Therefore, several specific Flt3-inhibitors have been developed and evaluated in clinical trials (for review see ref. 9). However, their overall clinical efficacy in AML must so far be considered as minor 9.

Sorafenib (Nexavar ™, formerly BAY 43-9006) has been approved for the treatment of metastatic renal cancer and advanced hepatocellular carcinoma 11. It inhibits the serine threonine kinase Raf-1, but also the Flt3-RTK, and Flt3-ITD 10, suggesting that may have a role also in AML 12. Sorafenib was recently tested in a phase I clinical trial on 16 patients with AML, and was found to be particularly active in six of the seven Flt3-ITD positive patients 10. However, treatment duration was short (21-70 days) and no durable responses were reported. Notably, a complete molecular remission has recently been reported in a patient relapsing after SCT 13. We here show, in a cohort of six Flt3-ITD-positive AML patients, that single agent sorafenib given on a compassionate-use basis led to notable long-term responses in refractory and relapsed disease prior and post allogenic stem cell transplantation (allo-SCT).
Material and Methods

Patients, treatment and objective
From November 2007 to November 2008 refractory or relapsed Flt3-ITD positive AML patients (n=6) prior or post allo-SCT were treated after informed consent at the University of Marburg with sorafenib on a compassionate-use basis. Treatment was performed in the absence of alternative therapeutic options outside a clinical trial. The initial dose was 2 x 400mg orally (p.o.) daily, and was adjusted in case of cytopenia, suspected toxicity or resistance (dose range: 200-800mg daily).

Response
Treatment response was monitored and remission was defined according to standard criteria: i) complete remission (CR): marrow blasts < 5%, neutrophils > 1 x10⁹/L, platelets > 100 x10⁹/L for at least 4 weeks; ii) bone marrow response (BMR): marrow blasts reduction by > 50% from start of sorafenib without hematologic recovery; iii) hematologic response (HR): disappearance of blasts from the peripheral blood; iv) complete molecular response (CMR): CR plus molecular negativity for Flt3-ITD by polymerase chain reaction.

Intracellular p-Stat-5 staining
Intracellular staining with phycoerythrin (PE)-conjugated anti-phospho-Stat-5 (Y694) was performed as previously described after informed consent was given, in accordance with the Declaration of Helsinki, according to a votum of the local ethics committee of the University of Marburg.
Results and discussion

**Sorafenib monotherapy in relapsed Flt3-ITD positive AML after allo-SCT**

Four patients (median age: 50 years; range: 42-62) with relapsed Flt3-ITD positive AML after allo-SCT were treated with sorafenib on a compassionate-use basis. The median time from allo-SCT to relapse was 192 days (range, 87 to 322 days). All patients had a normal karyotype. For treatment and response details see Table 1.

Patient #1 was a 62-year-old male with AML-M4 (Flt3-ITD-ratio: 11.4). After daunorubicin/cytarabine (DA) induction therapy and one consolidation cycle he underwent allo-SCT in first CR, but relapsed. He obtained a rapid HR and BMR under sorafenib. Due to leucopenia sorafenib was repeatedly paused and the sorafenib dose level was reduced. The patient died from AML-progression that was resistant to sorafenib on day +216 (Table 1).

Patient #2 was a 42-year-old female with normal karyotype AML-M4 (Flt3-ITD-ratio: 1.15). After two DA-induction cycles she underwent allo-SCT in first CR and relapsed despite experiencing a graft versus host disease (GvH) (intestine and skin). With sorafenib at 400mg p.o. bid, she obtained a rapid and sustained HR and BMR (Figure 1A, Table 1). Sorafenib inhibited the anti-apoptotic Flt3-ITD-target Stat-5 in vivo (Figure 1A). Concomitantly to an increasing donor chimerism (from 47% to 91%) GvH re-occurred, indicating that sorafenib did not compromise a GvL response. The patient died on day+221 of sorafenib, but remained essentially sorafenib-sensitive (Figure 1A).

Patient #3 was a 46-year-old female with Flt3-ITD positive AML-M2 (Flt3-ITD-ratio: 4.2). After two DA-induction cycles she underwent allo-SCT in first CR, but relapsed (Table 1). She also had experienced chronic GvH of the skin, joints, and poly-serositis.
requiring immune suppressive therapy with steroids. The relapse was treated with sorafenib 400mg p.o. bid and within two month a still ongoing CMR was achieved.

**Sorafenib facilitates allogenic stem cell transplantation in chemotherapy-refractory Flt3-ITD positive AML**

Three patients were rescued with sorafenib before allo-SCT. The median age of these patients was 40 years (range, 38–57 years).

Patient # 4 was a 56-year-old male with Flt3-ITD positive (Flt3-ITD ratio: 1.16) AML, who relapsed after one cytarabine consolidation cycle. The patient’s clinical status was poor. Under 400mg sorafenib, bid, he instantly achieved a HR (Table 1) and thrombopoiesis recovered in part (Supplemental Figure 1). Sorafenib inhibited Stat-5 activation in AML blasts in vivo, confirming in vivo the presumed mode of action of sorafenib via inhibiting anti-apoptotic targets of Flt3-ITD (Supplemental Figure 1).

Patient #5 was a 40-year-old female with AML-M5a (Flt3-ITD-ratio: 0.8) experiencing primary induction failure. Sorafenib was commenced and within 14 days of treatment a fast HR and BMR was seen (Figure 1B). The patient underwent allo-SCT using a dose-reduced conditioning regimen. She achieved a CR, but relapsed again on d+111 post allo-SCT. Sorafenib was re-started at 400mg bid, and a still ongoing CMR was obtained (Table 1).

Patient #6 was a 38-year-old male with primary chemotherapy-refractory AML-M2. He had the lowest Flt3-ITD-ratio in this cohort (0.39). A donor was not available and sorafenib was commenced at 400mg bid on an outpatient basis. A HR was achieved (Table 1). The patient relapsed on day +71 of sorafenib, but at this time allo-SCT could be performed. The patient remained in CR at last visit.
Here we support and significantly extend initial findings on the efficacy of sorafenib monotherapy in AML \textsuperscript{10,13}. Apparently, sorafenib has a consistent activity in relapsed and refractory Flt3-ITD-positive AML. With a median treatment duration of 158 days post allo-SCT (range, 90-221), only two patients developed sorafenib drug resistance, whereas two patients obtained an ongoing CMR. Based on these observations and the limited literature on sorafenib-monotherapy in AML, it is tempting to speculate that sorafenib may be most effective when applied in the context of an allo-SCT, because it has the potential to reduce leukemic burden, without compromising the restoration of a GvL response (pat #2, #3, and #5). This reminds on the successful scenario seen with pre-emptive imatinib treatment after allo-SCT in BCR/ABL+ acute lymphatic leukemia (Ph+ALL) \textsuperscript{17}.

However, sorafenib could also be a valuable compound to bridge the time to allo-SCT. It spared chemotherapy-toxicity, while still exerting anti-leukemic activity (pat. #5, #6). Equally important in this regard, circumventing ineffective cycles of chemotherapy prior to allo-SCT was shown to translate into better overall survival in high-risk AML \textsuperscript{16,18}. Notably, sorafenib did not adversely affect engraftment in patients #5 and #6. However, long-term sorafenib treatment was associated with neutropenia and thrombopenia in many patients. This is not typically seen during sorafenib treatment of renal or hepatic cancer \textsuperscript{11}, but may be explained by the compromised normal bone marrow reservoir in relapsed AML post allo-SCT.

Taken together, the role of sorafenib monotherapy in Flt3-ITD positive AML deserves evaluation in clinical trials. Based on its ability to induce CMR’s in relapsed patients after allo-SCT, a prophylactic treatment strategy after allo-SCT may hold the promise to markedly improve the poor outcome of Flt3-ITD positive AML.
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Authors contribution

A.B., A.N. and S.M. designed the research, analyzed the data, A.B. wrote the paper.

S.M., Y.W., M.W., S.T., and A.C. performed the in vitro research; A.B., E.W., S.M., and A.N. treated the patients. M.W., E.E., and M.E. contributed vital reagents and analyzed the data. None of the authors have declared any conflict of interest.
Literature

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Legend Table 1)

Abbreviations: SCT, allogenic stem cell transplantation; WBC, peripheral white blood cell count, HR, hematological remission, BMR, bone marrow remission; CMR, complete molecular remission, RIC, reduced intensity conditioning therapy.

# Patient was treated for only 14 days prior to SCT, when a donor became available and conditioning could be started. A hematological relapse occurred 111 days after SCT and the patient responded again to sorafenib (400mg bid), achieving a CMR.

† BMR is not according to ref. 14. Bone marrow was hypocellular (Figure 1b) but after only 14 days, nucleated cells consisted of still more than 50% blasts.

* according to the National Cancer Institute common toxicity criteria, version 2 (http://www.fda.gov/cder/cancer/toxicityframe.htm)
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Clinical status before sorafenib</th>
<th>Dose, mg</th>
<th>Treatment duration, days</th>
<th>WBC, x 10^9/L (blasts %) before sorafenib</th>
<th>Best response</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>after 7 days of sorafenib</td>
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<td></td>
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<td></td>
<td></td>
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<td>relapse day +87 post allo-SCT</td>
<td>200mg to 800mg</td>
<td>211</td>
<td>39.6 (78)</td>
<td>HR, BMR, neutropenia (IV) thrombopenia (IV) pneumonia hemolysis</td>
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<tr>
<td>2</td>
<td>relapse day +168 post allo-SCT</td>
<td>200mg to 800mg</td>
<td>221</td>
<td>73.5 (84)</td>
<td>HR, BMR, neutropenia (IV) thrombopenia (IV)</td>
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<td>3</td>
<td>relapse day +322 post allo-SCT</td>
<td>800mg</td>
<td>81</td>
<td>18.6 (80, in BM)</td>
<td>CMR, CR, maculo-papulous exanthema of the skin (GvH)</td>
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<tr>
<td>4</td>
<td>relapse d+30 after first cons.</td>
<td>400mg to 800mg</td>
<td>50</td>
<td>44 (91)</td>
<td>HR, BMR, neutropenia (IV) thrombopenia (IV)</td>
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<tr>
<td>5</td>
<td>primary refractory</td>
<td>800mg</td>
<td>13*</td>
<td>36.2 (94)</td>
<td>HR BMR†, CMR, neutropenia (IV) thrombopenia (III) sepsis</td>
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<tr>
<td>6</td>
<td>primary refractory</td>
<td>800mg</td>
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<td>8 (26)</td>
<td>HR, hand-foot-syndrome hyperkeratosis</td>
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<th>Emergence of sorafenib resistance</th>
<th>Outcome at last visit on sorafenib (grade*)</th>
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<tr>
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<td>exitus letalis on day +216 in relapse</td>
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<tr>
<td>no</td>
<td>exitus letalis on day +221 with cerebral mass, neutropenia (IV) thrombopenia (IV)</td>
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<tr>
<td>yes</td>
<td>relapse letalis on day +58 in sepsis, neutropenia (IV) thrombopenia (IV)</td>
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<tr>
<td>no</td>
<td>relapse letalis on d+111 after allo-SCT, sorafenib monotherapy 400mg bid =&gt; ongoing CMR</td>
</tr>
<tr>
<td>yes</td>
<td>relapse on d+71 RIC-allo-SCT =&gt; CR</td>
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</table>
Figure Legends

Figure 1)

Sorafenib treatment response

A) Patient #2: A fast response to sorafenib was associated with a dephosphorylation of the anti-apoptotic Flt3-ITD target Stat-5, measured intracellularly in the blastic population by FACS (middle panel histogram): the left shift of the green curve documents a decrease in intracellular Stat-5 phosphorylation 7 days after commencing sorafenib compared to baseline (day 0, red line). Sorafenib was withdrawn on day 204 of sorafenib and hydroxyurea commenced because of a newly diagnosed cerebral mass. AML was resistant to hydroxyurea (WBC count increase to $75.6 \times 10^9/\text{L}$), but re-exposure to sorafenib again led to an instant response (WBC decline to $1.3 \times 10^9/\text{L}$). B) Patient #5: Bone marrow light microscopy to evaluate bone marrow response (patient #5) before allo-SCT. Giemsa staining shows blast infiltration prior (left panels) and extensive blast clearance 14 days after commencing sorafenib. Right panels: low and high magnification of bone marrow smear (upper and lower panels, respectively).